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Research Article

Standardization of leaves of *Ziziphus nummularia* Linn. - An effective herb for UTI infections

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Abstract

Ziziphus nummalaria Linn. (Syn. *Z rotundifolia*) (Rhammaceae), commonly known as jhar ber is a perennial herb used in the treatment of various disease and disorders of human and animal ailments. The present work was conceived to standardize the leaves. The leaves were extracted with water, methanol and petroleum ether. Further phytochemical screening was performed which revealed the presence of carbohydrates, protein, alkaloids, saponins and glycosides. Also physicochemical analysis of powdered leaves such as determination of ash value, extractive value, FOM and swelling index are reported in present paper.

Keywords: *Ziziphus nummalaria,* Leaves, Phytochemical screening, Standardization

Introduction

Medicinal plants are an important therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century[1]. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms.[2] The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants.[3] To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore.[4-5]

Ziziphus nummularia, also called jhar ber, is species of ziziphus native to the western India and southeastern Pakistan and south Iran. It is a shrub upto 2 meter high, branching to form a thicket. The leaves are simple, shining green above and whitish tomentose beneath, due to persistent dense hairs (occasionally glabrous), margins minutely serrulate, leaf shape ranging from almost round to an elongated ellipse, commonly sub-orbicular to ovate-oblong, rounded at both ends, highly variable in shape and size but always with three basal nerves and two stipular spines, one long and straight, the other small and curved back, and often brown in colour. The leaves are petiolate, 1.1 –5.8mm long and stipules are mostly spines, in each pair one hooked and one straight. The bark is reddish brown with long vertical fissures, reddish and fibrous inside. The branches are spreading and droop at the ends. *Ziziphus* belongs to the kingdom; plantae, order; roasles, division; magnoliophyta, class; magnoliopsida, family; rhamnaceae, genus; *ziziphus*.[6-12]

Urinary tract infections (UTIs) are a leading cause of morbidity and health care expenditures in persons of all ages. Sexually active young women are disproportionately affected, but several other populations, including elderly persons and those undergoing genitourinary instrumentation or catheterization, are also at risk. An estimated 40 percent of women report having had a UTI at some point in their lives. Urine located within the urinary tract, excluding the distal region of the urethra is considered sterile in healthy individuals, as indicated by the absence of cultivable bacterial cells.

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A urinary tract infection (UTIs) describes a condition in which there are micro organisms established and multiplying within the urinary tract. It is most often due to bacteria (95%), but may also include fungal and viral infection[13-16]. It was experimentally found that leaves of *Ziziphus nummmlaria* have prompt action toward Pathogen causing UTI Infection In the present study various standardization parameters of leaves of *Ziziphus nummmlaria* plants were evaluated and are presented.

Material and Methods

Plant materials

The leaves of *Ziziphus nummalaria* Linn. were collected from Malwa region of Madhya Pradesh in the month of Feb-March, 2007 and were identified by the Botany Department, Janata PG College, A.P.S. University, Rewa (M.P.) and was deposited in our department (Voucher specimen No. Z-33/05). The leaves were later air-dried, powdered and stored in an air-tight container for further use.

Physico-chemical evaluation

The dried leaves of *Ziziphus nummalaria* Linn., were subjected to standard procedure for the determination of various physicochemical parameters[17-18]. The results are presented in Table 1.

Determination of ash values

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Total ash value

Accurately about 3 gms of air dried powder of leaves of Ziziphus nummalaria *Linn.* was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450^{0}C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

Acid insoluble ash

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Determination of moisture content (Loss on drying)

Place about 10 g of drug (without preliminary drying) after accurately weighing in a tared evaporating dish and kept in oven at 105^0 C for 5 hours and weigh. The percentage loss on drying with reference to the air dried drug was calculated.

Determination of foreign organic matter

Accurately weighed 100 g of the drug sample and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and the percentage present was calculate.

Determination of swelling index

Swelling index is determined for the presence of mucilage in the seeds. Accurately weigh 1 g of the seed and placed in 150 ml measuring cylinder, add 50 ml of distilled water and kept aside for 24 hours with

occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured.

Preparation of extracts[11-12]

5g of the leaf powder of *Ziziphus nummalaria* Linn. was extracted with petroleum ether (40 – 60^{0}C), methanol and water in soxhlet apparatus. The different extracts were tested for the presence of steroids, reducing sugars, carbohydrates, triterpenoids, alkaloids, phenolic compounds, saponins, protein glycosides, tannins and flavonoids. The phytochemical tests were performed and the results obtained are presented in Table 2.

Preliminary phytochemical screening[19]

Preliminary phytochemical analysis of the various extracts of the leaf powder in various solvents has been performed. The results obtained are presented in Table 3.

Fluorescence analysis

The leaf powder of *Ziziphus nummalaria* Linn. was dried under shade and powdered. The leaf powder and the extracts of the powder in various solvents were examined under ordinary light and in UV-light (nm). The fluorescence characters were determined according to the methods of Chase and Pratt[20]. The results obtained are presented in Table 4.

Results and discussion

In this study the results of the investigations show the standardization parameters of leaves of *Z. nummalaria* Linn. Various pharmacognostical studies of the powdered leaves such as physicochemical parameters, preliminary phytochemical screening and fluorescence analysis were evaluate. Various quantitative determinations such as percentage of loss of weight on drying, total ash, water-soluble ash, acid insoluble-ash. Swelling index and FOM were evaluated (Table 1). The percentage of extractive values in petroleum ether (40– 60^{o}C), benzene, methanol and distilled water were also determined (Table 2).

The leaf powder of *Z. nummalaria* Linn. and the extracts of the powder in various solvents were examined under ordinary light and UV light (365 nm). The powder was also treated with various chemical reagents and the changes in colour were recorded (Table 3).

The preliminary phytochemical screening (PPS) of all the three extracts were performed which shows the presence of various active phytoconstituents (Table 4).

Therefore, it was concluded from the present investigation that the selected species of *Ziziphus* contains various active phytoconstituents which was confirmed by preliminary phyctochemical screening. Hence, detailed screening may be formed to isolate active moiety so, that it may be scientifically proved to access the pharmacological responses of the plant to ascertain its folklore uses.

Thus, the fluorescence analysis, physico–chemical evaluation and preliminary phytochemical screening can be used as a diagnostic tool for the correct identification of the selected species of Ziziphus. Hence, these standardization parameters are useful in detecting the adulterants if any in this plant and will lead to efficacy and purity of the selected plant.

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Table 1: Physicochemical parameters of leaves of *Z. nummalaria* Linn.

Parameters	Values obtained
Total ash	3.6%
Water soluble ash	1.7%
Acid insoluble ash	0.8%
Swelling index	12.4%
FOM	2.4%

Table 2: Extractive values of leaves of *Z. nummalaria* Linn.

Type of extract	% yield (w/w)	Color of extract
Aqueous extract (Distillled Water)	16.7	Reddish brown
Methanolic	14.2	Dark Brown
Benzene	3.2	Greenish Brown
Pet. Ether extract	8.9	Dark black

Table 3: Fluorescence characters of *Z. nummalaria* Linn. leaf powder and their extracts in different solvents

S/No.	Solvents	Visualized color Under	
		Ordinary Light	UV light (365nm)
1.	Hot water	Pale yellow	Light orange with blue fluorescence
2.	Cold water	Light green	Light orange with blue fluorescence
3.	Normal water	Light green	Light orange with blue fluorescence
4.	Chloroform	Green	Yellow with purple border
5.	Ethanol	Green	Reddish orange
6.	Methanol	Dark green	Reddish orange
7.	n-Hexane	Light green	Dark orange
8.	Pet. Ether	Green	Dark orange
9.	n-butanol	Green	Very light orange
10.	Ethyl acetate	Dark green	Very light orange
11.	Toluene	Green	Dark reddish orange
12.	Benzene	Green	Dark reddish orange
13.	Ether	Dark green	Dark reddish orange
14.	n-Hexane	Colorless	Blue light

Table 4: Preliminary phytochemical screening of leaves of *Z. nummalaria* Linn.

Phytoconstituents	AE	ME	PEE
CHO	+	+	+
Protein & AA	+	+	+
Glycosides	-	+	+
Alkaloids	-	+	-
Terpenoids	-	-	-
Fixed oil	-	-	-
Wax & Fats	-	-	-
Saponins	+	+	+

Abbr.: AA= Aqueous extract; ME=Methanolic extract; PEE=Petroleum Ether Extract, + Present; -Absent